Gut permeability measured by polyethylene glycol absorption in abnormal gut fermentation as compared with food intolerance

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SUMMARY

Gut permeability has been studied in patients with either food intolerance or abnormal gut fermentation as well as in normal subjects. Permeability was measured by polyethylene glycol absorption, and the reasons for this choice of probe are discussed. Results show that both symptomatic groups have statistically very highly significant deviations from the normal ($P<0.01$), consisting of over-absorption, significant at molecular weights 242, 286, 330 and 374. Whilst both study groups were different from the normal they were not different from each other. The implications for these findings in the diagnosis and management of food intolerance and abnormal gut fermentation are discussed.

INTRODUCTION

Abnormal gut fermentation (AGF) as a syndrome synonomous with germ-carbohydrate fermentation and intestinal carbohydrate dyspepsia has been with us for many years. *Candida albicans* is causally suggested in a similar syndrome, but without evidence for this[1]. The justification of AGF as a nosological entity rests on a novel test published in 1991[2,3], which consists of a loading dose of orally administered glucose following a fast with subsequent blood alcohol estimation after 1 h continued fast. An abnormality is held to exist when the level of ethanol exceeds 22 µmol/l. The validity of the test has been confirmed in other laboratories (Brostoff, personal communication, 1993). AGF thus defined is a diffuse symptom complex which is not distinct from other diagnoses: it may co-exist in patients who have irritable bowels, often but not invariably accompanied by psychological, catarrhal and general symptoms[1]. Management embraces the use of a diet low in fermentable, yeasty and mouldy foods with or without antifungal drugs. In successfully treated patients the gut fermentation alcohol test reverts to normal in most cases.

Food intolerance is also thorny ground. At first we sought it in catarrhal symptoms[4], but it has been found in patients with irritable bowel[5] and psychological symptoms[6]. The symptom complex in food intolerance may thus be no different from that seen in AGF. The basic diagnostic tool is that of elimination and challenge diet: suspect foods are omitted for a period. Symptoms clearing and recurring on food re-introduction is diagnostic[7]. However, other factors, such as aversion could give the same result[7]. Laboratory testing has not so far proved reliable nor has an immune basis been demonstrated. Consequently, some have advocated double blind food challenges, which however are also problematic[7]: the food dose sufficient to cause symptoms varies, not all foods can be 'hidden', and food intolerance, unlike allergy, may remit on avoidance. An open elimination and challenge programme may take up to 9 months. By the time double blind repeats are added sensitivity may have been lost: a false negative. It may be wisest to accept the limits of the available diagnostic facilities and use solely an open technique with all its imperfections.

The pool of patients presenting to a 'working allergist' is selective and differs from those presenting to other subspecialties. Most of these patients have had multiple previous referrals, with negative findings, and patients with established diagnoses of inflammatory bowel disease, gall stones or peptic ulceration, etc., have been excluded. Psychological problems are a dominant co-factor, necessitating one-third of this group receiving some form of psychological counselling, but the precept is followed of always seeking for reproducible physical findings (such as laboratory tests, not all dealt with in this study) before considering a psychiatric diagnosis.

A previous study shows low body levels of B vitamins, zinc and magnesium in patients with AGF[8], a finding which is not likely to be due to any dietary deficiency, but may well relate to altered gut permeability. The purpose of this

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study was to assess the relationship of intestinal permeability to patients with AGF or food intolerance.

SUBJECTS AND METHODS

Patients

Successive referrals to one of the authors (KKE), when the diagnoses of AGF or food intolerance were suspected were subjected to laboratory testing for abnormal alcohol and polyethylene glycol (PEG). The results were evaluated at a subsequent consultation. As detailed in the introduction, the clinical presentation may be similar in either condition. The patients who have food intolerance, however, may be distinguished from gut fermenters by lack of clinical response to a diet low in fermentable foods and the concurrent administration of antifungal drugs in those who do not respond to diet alone. By contrast, patients with gut fermentation may respond to an oligo-antigenic diet, as it excludes fermentable foods. Both conditions may co-exist in the same patient: at present this can only be established by an incomplete response to measures to control one of the two entities and not the other. The diagnosis of food intolerance was established in alcohol negative patients by elimination and challenge dieting[7]. Patients in whom follow up was maintained and who had abnormal alcohol in PEG tests were retested at intervals of not less than three months.

Gut fermentation alcohol testing

Detail of testing technique and methodology are reproduced elsewhere[2,3]. The clinical test involves abstaining from alcoholic intake for 24 h and a fast for 3 h prior to testing. The patient is then administered a 1g glucose challenge in hardened gelatine capsules together with 100 ml of 4% glucose solution. The capsuled glucose is expected to pass into the duodenum. The dose employed was determined as a result of practical trials using various dosage levels. The fast is maintained until the blood sample has been drawn 1 h after capsule ingestion.

Polyethylene glycol

On the morning of the test on rising, the patient empties the bladder and takes a 1.00g test dose of PEG 400 diluted in fruit juice whilst still fasting. Food is thereafter taken normally. All urine is collected for 6 h. No allowance has been made for the osmolarity of the test solution. The two tests were performed separately.

Analytical method for PEG 400 A Perkin-Elmer F33 gas-liquid chromatograph was equipped with a 2.4 m 3.175 mm glass column packed with Chromosorb 108, 100/120 mesh, (Sigma Chemical Co. Ltd, Poole, UK). The stationary phase and general conditions were according to the original method by Chadwick *et al.*[13], but the following modifications were made:

Fifty microlitres of the internal standard (pentaerythritol) was added to a 5 ml aliquot of the urine collection, 1 ml of the acetylation mixture and one-fifth of the other reagent volumes were used.

A comparison was made between the original method of PEG isolation and a solvent extraction procedure. (Details can be obtained from JMH and this data will form the basis of a future paper on the measurement of PEG in urine.)

The carrier gas was argon with a flow rate of 25 ml/min. The flame ionization detector was set up according to the manufacturer's instructions and then the hydrogen flow was increased by 18%. This gave the best reproducibility with only a very slight loss of sensitivity.

Two microlitres of the final acetone solution of the extracted PEG was injected onto the column with the temperature maintained at 180 °C. After a 5 min isothermal stage the temperature was increased by 10 °C/min to a maximum of 380 °C.

The internal standard peak appears first at around 200 °C and it is followed by the PEG 198 peak about 1 min later. The other 10 peaks, up to molecular weight (MW) 638 are spread evenly over the next 15–16 min. The peak areas of the internal standard and test peaks, together with the urine volume, were used in calculating the results. The total integral peak area was better than peak height measurements.

The composition of the PEG 400 was measured in quadruplicate using the above technique and further checks were made at frequent intervals during the life of the batch.

The percentage of the ingested dose excreted for each MW from 198 to 638 was calculated.

Reproducibility was excellent with a coefficient of variation (CV) of 1.7% for a single sample analysed on 30 successive working days. The CV was calculated for the total PEG excreted. Initially all samples were analysed in triplicate, but with further experience single analytical runs were used provided the peak area of the internal standard was within 5% of the expected value. Abnormal test results were repeated and the duplicate results were almost invariably within 4% of each other. A triplicate analysis always brought the reproducibility within 4%.

RESULTS

The normal baseline for the study was 50 well individuals, without any complaints, and not on any medication or nutritional supplements. The normal range was compiled to range from the lowest to the highest figures recorded at each molecular weight (MW). It does not statistically differ very significantly from ± 2 standard deviations from the mean, but it is a little higher.

Table 1 **Comparison of mean polyethylene glycol (PEG) values in normal and food intolerant (FI) patients**

	PEG198	PEG242	PEG286	PEG330	PEG374	PEG418	PEG462	PEG506	PEG550	PEG594	PEG638	TOTAL
r	0.3487	0.4611	0.3834	0.5162	0.4626	0.4033	0.3077	0.1437	0.0355	0.0175	0.0117	
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.138	0.715	0.858	0.904	
Group 1 Mean (SD)	30.9 (2.1)	29.3 (1.7)	27.4 (1.3)	23.5 (1.3)	20.1 (1.2)	14.3 (1.1)	8.6 (1.3)	4.8 (0.7)	1.6 (0.4)	0.7 (0.4)	0.3 (0.2)	
Group 2 Mean (SD)	31.7 (2.2)	30.8 (2.9)	28.5 (5.2)	26.4 (4.2)	22.9 (4.1)	16.7 (4.4)	10.6 (4.1)	5.8 (3.2)	2.4 (2.7)	1.3 (2.1)	0.6 (1.6)	13.5 (3.3)
Group 3 Mean (SD)	32.6 (0.8)	31.7 (1.4)	30.4 (1.8)	27.3 (2.9)	23.3 (2.6)	17.3 (3.2)	10.5 (2.4)	5.4 (1.0)	1.6 (0.5)	0.7 (0.4)	0.3 (0.3)	13.6 (1.8)

Group 1=Normals (n=50); group 2=GFP negative and FI (n=29); group 3=GFP positive and FI (n=29); r=Pearson's correlation between the group numbers and the PEG recovered in urine

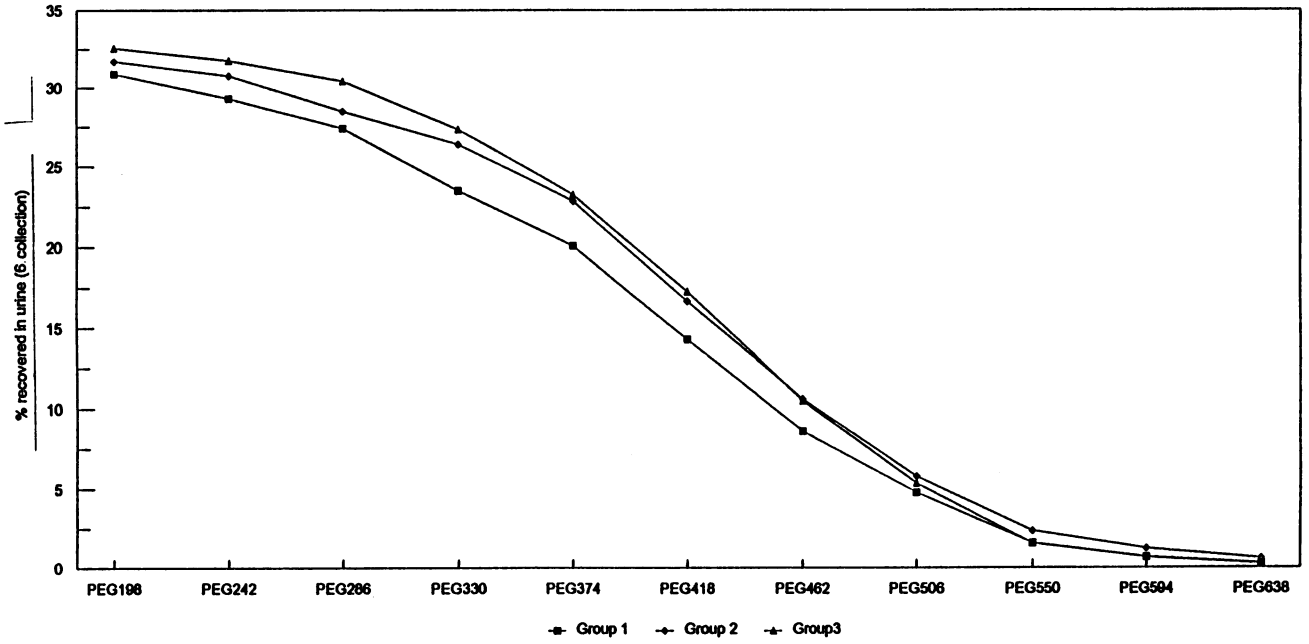

Figure 1 **Mean polyethylene glycol (PEG) values**

Twenty-nine alcohol positive subjects were identified and 29 subjects with food intolerance. In 21 of 29 alcohol positive subjects, and in 24 of 30 of the food intolerant group the PEG test was classed as abnormal (one or more MW fractions outside of the limits established for the 50 control individuals). In retrospect, we should not have taken a single abnormality as constituting abnormal status, as statistical prediction for a normal range of $\pm$two standard deviations would give a single figure outside the reference range once in every 20 subjects. However, in practice no individual had only one abnormal MW finding. The minimum for those with abnormalities was two, the maximum 10, with means of 5.2 for group 2, and 4.7 for group 3. However, these abnormalities were statistically significant only at MW 262, 286, 330 and 374.

As stated in the introduction the presenting symptoms of both conditions currently overlap. The diagnoses were made on the basis of gut-fermentation alcohol tests (for AGF) or elimination and challenge dieting (for food intolerance). However, it is desirable to know how reliable this is in clinical terms, and whether prior to or subsequent to being included in this series the opposite diagnosis was made. The notes of all subjects were therefore reviewed 2 years after completion of the project to ascertain whether the contra diagnosis was also involved. In the alcohol positive group follow-up was maintained in 25 of 29 patients. Seven had the alternate diagnosis made of food intolerance. In the alcohol negative, diet and challenge positive group follow-up was preserved in 21 of 29. Six had an alternate diagnosis of AGF.

For the two symptomatic groups, those with food intolerance, and those with AGF, the data are summarized in Table 1 and Figure 1. It will be seen that both symptomatic groups differed markedly from the normal, but not from each other. When the data were analysed, using a non-parametric 2×2 table, the results were very highly statistically significant (P<0.001).

DISCUSSION

The total urinary excretion of PEG 400 was slightly higher than in previously published studies[13]. Since the present test concerns comparisons this does not invalidate the findings we present. The present study reports statistically very highly significant deviations of gut permeability from normal in gut fermentation and food intolerance. The significant differences were however only present at MW 242, 286, 330 and 374 (in future studies it might be worth pre-selecting appropriate MW). Gut fermentation is, in relation to short chain fatty acids, a normal phenomenon responsible for the colonic bacterial digestion of soluble fibre[16]. Because of the timing of the gut fermentation alcohol test with blood sampling 1 h after sugar challenge we presume that we are looking at an activity of the small bowel. However, the ethanol amounts produced are small, and probably too low to be directly the cause of symptoms, although clearly the as yet unidentified causative organism might do so. Nevertheless there are also abnormalities of vitamin and mineral absorption[8] and this current work shows abnormalities of PEG absorption similar in both range and severity to food intolerance, an established cause of morbidity. In both groups the changes were only statistically significant at MW up to 506: in food intolerance it would have been expected that they were at the higher weights, although clinically the authors find vitamin and mineral handling problems in these patients also, and therefore perhaps all the abnormalities seen in both groups may relate to micronutrient malabsorption. As we saw over-absorption, rather than under-absorption perhaps some competitive mechanism is responsible? A deliberate decision was made to include both entities in this study, as at present the symptom complex associated with gut fermentation is ill defined[1], and there is considerable clinical overlap with food intolerance. A number of authors have suggested increased gut permeability as a mechanism in food intolerance[17–19]. If partly digested foods are absorbed an adverse immunological reaction is to be expected: this could well be a mechanism for food intolerance. We have been able to confirm that the event does occur in a majority of patients in this study.

Gut permeability may be increased in moderate alcoholics who are on an apparently normal diet[20], although this finding is at present controversial. The gradual improvement recorded on follow-up studies indicates a future for better defined management programmes which themselves need to be the subject of adequate double blind trials. It is clearly desirable that our findings should be repeated with the inclusion of other techniques of assessing gut function. Accordingly, a collaborative study has been initiated to compare the modified PEG technique as used in this study with the double sugar test (lactulose/L rhamnose), 3-0-methyl-D-glucose and D-xylose.

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